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L1: Entry 1 of 1 File: USPT Jul 29, 2003

DOCUMENT-IDENTIFIER: US 6599527 B1
TITLE: Preparation of pharmaceutical compositions

Brief Summary Text (16):
A well-recognised approach to the formulation of lipophilic drugs is liposome encapsulation in which the drug is intercalated into the lipid bilayer(s) of the liposome. Compositions, methods of preparation, applications, advantages and disadvantages of liposomes have all been extensively reported, and there are more than 30 publications describing liposomal entrapment of CyA mainly for intravenous and systemic use.

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L3: Entry 27 of 52 File: USPT Jan 2, 2001

DOCUMENT-IDENTIFIER: US 6168778 B1
TITLE: Vascular endothelial growth factor (VEGF) Nucleic Acid Ligand Complexes

Detailed Description Text (41):
During Liposome formation, water soluble carrier agents may be encapsulated in the aqueous interior by including them in the hydrating solution, and lipophilic molecules incorporated into the lipid bilayer by inclusion in the lipid formulation. In the case of certain molecules (e.g., cationic or anionic lipophilic drugs), loading of the drug into preformed Liposomes may be accomplished, for example, by the methods described in U.S. Pat. No. 4,946,683, the disclosure of which is incorporated herein by reference. Following drug encapsulation, the Liposomes are processed to remove unencapsulated drug through processes such as gel chromatography or ultrafiltration. The Liposomes are then typically sterile filtered to remove any microorganisms which may be present in the suspension. Microorganisms may also be removed through aseptic processing.

L3: Entry 40 of 52 File: USPT Sep 17, 1991

DOCUMENT-IDENTIFIER: US 5049392 A
** See image for Certificate of Correction **
TITLE: Osmotically dependent vesicles

Brief Summary Text (11):
In the dried film MLV technique of Bangham et al described above, hereinafter referred to as the Classical Method), lipids are dissolved in a suitable solvent, the solvent rotoevaporated to form a dry lipid film on the flask and the dry film hydrated with an aqueous medium. Lipophilic drugs are incorporated into the liposome by co-dissolving them in the solvent phase while aqueous soluble materials are entrapped from the hydration buffer. While such technique of drug encapsulation is advantageous in that there are not required disruptive applications of heat, sonication, freezing or the addition of solvents (which can facilitate degradation, denaturation, or inactivation of many drugs, especially proteins), a number of disadvantages do exist. In the first place, the resulting product tends to be unstable both in terms of leakage of drug from the capsule into the external aqueous environment and in terms of the presence of oxidation or lyso products. This instability has been attributed to the uneven distribution of drug in the vesicle. More specifically, it has been found that in the onion-like MLVs, the encapsulated drugs tend to be present in high concentrations in the center of the MLV but at low concentrations at the outer layers of the MLV. This concentration differential creates a state of osmotic non-equilibrium and destabilizes the vesicle. Another problem with MLVs prepared by the Classical Method is that only small amounts of drug are sequestered therein, i.e., only between about 5 and 10% of the drug present in the initial solution. This is highly disadvantageous especially when encapsulating very expensive drugs. Yet another problem with the Classical Method is that the formulation of the films along the walls of the reaction vessel renders it difficult to adapt the process to large scale production techniques.

L3: Entry 44 of 52 File: USPT Jun 11, 1991

DOCUMENT-IDENTIFIER: US 5023087 A
TITLE: Efficient method for preparation of prolonged release liposome-based drug delivery system

Detailed Description Text (34):
The compounds entrapped in the liposomes are a liposome impermeable and/or water soluble drugs such as peptides, antibiotics, hormones and other drugs whose rate of diffusion out of liposomes is not significantly greater than the rate of breakdown of liposomes at an IM site of injection. The encapsulated drug may be a lipophilic drug or hormone whose oil/water partitioning strongly favors the liposome bilayer phase, or a water-soluble drug or peptide which is capable of diffusing across the liposomal bilayer slowly, if at all. Specifically excluded from the invention are all lipophilic or water-soluble drugs which can freely diffuse out of liposomes with a half-life of less than two hours.

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L1	lipophilic adj3 (drug or active) adj5 (liposom$) adj5 encapsul$	1	L1

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L1: Entry 5 of 14 File: USPT Jan 4, 2000

DOCUMENT-IDENTIFIER: US 6010890 A
TITLE: Method for viral inactivation and compositions for use in same

Detailed Description Text (28):
The usefulness of liposomes as a delivery vehicle for Pc4 as a photosensitizer for inactivation of lipid-enveloped viruses in RBCC was assessed using VSV as a model virus. FIG. 1 shows that the rate of inactivation of VSV does not depend on time of incubation of liposomal Pc4 prior to light exposure and that it is similar to that obtained when Pc4 is delivered in an organic solvent (DMSO) or in a detergent-like agent (Cremophor). However, binding of Pc4 to red cells does depend on the delivery vehicle and, in the case of liposomes, on the pre-irradiation incubation time (Table 1). These results suggested that exposure to light after short incubation times with liposomal Pc4 should result in less damage to red cells. FIG. 2 shows this is indeed the case. Treated red cells were stored and their hemolysis followed, and as expected from the binding studies, formulation of Pc4 in liposomes resulted in less hemolysis, and incubation for 5 minutes was better than for 30 minutes.

L1: Entry 7 of 14 File: USPT Jun 16, 1998

DOCUMENT-IDENTIFIER: US 5766624 A
TITLE: Liposomal defensins

Drawing Description Text (13):
FIG. 11. Effect of Different Lipids on Red Blood Cell Hemolysis. DSPC/Chol and DSPC/Chol/DDAB liposomes were prepared and used in hemolysis assays to measure the effect of the indicated lipids on RBC hemolysis. The results are presented as the percent hemolysis (relative to zero and one hundred percent hemolysis controls) induced at various lipid concentrations in the RBC samples.

Drawing Description Text (14):
FIG. 12. Hemolytic Activity of Indolicidin/DOPE Liposomes. Indolicidin /DOPE liposomes were prepared and used in hemolysis assays. The results are presented as the percent hemolysis (relative to zero and one hundred percent hemolysis controls) induced at various lipid concentrations.

Detailed Description Text (84):
Indolicidin-containing POPC and DPPC liposomes were prepared in accordance with the procedures described above, but without the freeze-thaw cycles, or in the case of interdigitation-fusion (IF) vesicles, with the procedure disclosed in (Janoff et al., U.S. Ser. Nos. 07/961,277 and 08/066,539, filed Oct. 14, 1992 and May 24, 1993, respectively, the contents of which are incorporated herein by reference). Red blood cell (RBC) samples were combined with these liposomes, and the degree of hemolysis induced was measured.

Detailed Description Text (85):
The hemolysis assay used measures the level of hemoglobin in the supernatant of RBC samples, the amount of hemoglobin released to the supernatant being indicative of the damage to red blood cell membranes induced by liposomal defensins. The hemolysis assay employed phosphate buffered saline (PBS), human red blood cells, polystyrene tubes and disposable cuvettes designed for use in UV spectrophotometers. Approximately 3 ml of packed RBCs was placed in a 15 ml tube, to which 10 ml of PBS was added. The RBCs were suspended, and the suspension was centrifuged for 10 min. at 4,000 rpm. The supernatant above the pellet was discarded, and more PBS was added. This washing process was repeated until the supernatant was about clear. Two ml of the final RBC pellet was suspended in 48 ml of PBS. The resultant RBC suspension was divided amongst a set of test tubes (0.5 ml RBC suspension per tube), to which were added additional buffer and POPC/indolicidin or DPPC/indolicidin liposomes. The tubes were capped, vortexed and then incubated for 20 hours on an agitator in a 37 deg. C. incubator. After this incubation, the tubes were centrifuged at low speed (<3000 rpm) for 10 minutes. An aliquot (0.2 ml) of the supernatant from each tube was placed in a cuvette to which was added 1.0 ml of water. Hemoglobin levels in the supernatants were determined by measuring absorbance at 550 nm, and are given as percent hemolysis relative to controls. The zero percent hemolysis control comprised RBCs and HEPES buffer (the same buffer composition in which the indolicidin-containing liposomes were suspended); the one hundred percent hemolysis control comprised RBCs and distilled water.

Detailed Description Text (86):

FIG. 8 presents the percent hemolysis induced by free indolicidin (open squares), as well as by POPC/indolicidin liposomes (open triangles), DPPC/indolicidin liposomes (open circles) and DPPC/indolicidin interdigitation-fusion (IF) liposomes (filled circles). The data shows that entrapment in liposomes lessens indolicidin's hemolytic activity, i.e., there is a reduction in the percent of hemolysis, in comparison to the free form of the defensin.

Detailed Description Text (94):
Hemolysis assays were conducted as described above (see Example 14). The results (see Table 5, below) indicate that increasing the indolicidin (8.27 mole % in DPPC-containing liposomes) levels (mg/ml) in the RBC samples resulted in an increased level of hemolysis.

Detailed Description Text (98):
Initial and final indolicidin concentrations were measured by absorbance at 280 nm. Initial and final lipid concentrations were determined by a standard phosphate assay (see Chen et al., Anal. Chem. 28:1956 (1956)). The liposomes were used in hemolysis assays, conducted in accordance with previously described procedures (see Example 14). The data (see FIG. 10 and Table 6) is presented as the percentage of hemolysis induced by free indolicidin (filled squares) as well as the percent hemolysis induced (filled circles) by the DSPC/Chol/indolicidin liposomal formulation, at various indolicidin concentrations in the RBC samples.

Detailed Description Text (101):
DSPC/Chol and DSPC/Chol/DDAB liposomes were prepared by dissolving the lipids in chloroform and then adding methanol, using two volumes of methanol per volume of chloroform, so as to form a monophase (see Fountain et al., U.S. Pat. No. 4,588,578). Two 0.5 ml portions of HEPES buffer (10 mM HEPES, 150 mM NaCl, pH 7.4) were added separately, with swirling after each addition. The samples were rotoevaporated at room temperature, and then at 60 deg. C., to remove the solvent. The dried samples were rehydrated with HEPES buffer to a final volume of 4 ml so as to form multilamellar liposomes having substantially equal interlamellar solute distribution. These liposomes were combined with RBC suspensions, and hemolysis assays were conducted according to procedures described above (see Example 10) to measure the hemolytic properties of the lipids. The data (see FIG. 11 and Table 7, below) shows the percent hemolysis induced at various lipid concentrations, with no indolicidin in the preparations.

Detailed Description Text (104):
DOPE/indolicidin liposomes were prepared by drying an indolicidin/DOPE/organic solvent solution, in a round-bottom flask, by rotoevaporation. The dried lipids were resuspended in 2 ml of HEPES buffer (10 mM HEPES, 150 mM NaCl). A free (unentrapped) indolicidin control was prepared by dissolving 2 mg of indolicidin in 1 ml of HEPES buffer. Lipid concentrations were determined by phosphate assay (see Chen et al., Anal. Chem. 28:1956 (1956)); indolicidin concentrations were determined by measuring absorbances at 280 nm. The data (see FIG. 12 and Table 8, below) show that hemolysis generally increased with increasing indolicidin concentration, both for the liposomal and free forms of indolicidin, and that at approximately the same concentrations in the RBC samples, indolicidin entrapped in DOPE liposomes induced about the same percentage of hemolysis as did unentrapped indolicidin.

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L1: Entry 10 of 14 File: USPT Mar 6, 1990

DOCUMENT-IDENTIFIER: US 4906477 A
TITLE: Antineoplastic agent-entrapping liposomes

Brief Summary Text (33):
Further, the membranes of the antineoplastic agent-entrapping liposomes according to the invention are stable in the presence of saline or 75% human serum. The liposomes have also the following advantages: they do not cause hemolysis and do not induce platelet aggregation in vitro. When adriamycin-entrapping liposomes are given to mice, a concentration of the drug in the blood is kept at higher level than that in administration of free adriamycin. The accumulation in the spleen is higher, too, in the administration as the drug-entrapping liposomes. On the other hand, drug accumulation in the heart and kidney is lower, so that cardiotoxicity and nephrotoxicity might be reduced. Therefore, the present invention provides the liposomes that can entrap the antineoplastic agent at high yield, reduce toxicity in dosing, and might be safely used as drug carriers.